UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/009,782	03/25/2002	Ken-Ichi Takeuchi	217301US0PCT	1319

22850 7590 02/21/2003

OBLON, SPIVAK, MCCLELLAND, MAIER & NEUSTADT, P.C.
1940 DUKE STREET
ALEXANDRIA, VA 22314

EXAMINER	
WALICKA, MALGORZATA A	

ART UNIT	PAPER NUMBER
1652	7

DATE MAILED: 02/21/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/009,782	TAKEUCHI ET AL.	
	Examiner	Art Unit	
	Malgorzata A. Walicka	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period f r Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *25 March 2002* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a)☐ The translation of the foreign-language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *3* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

The application is the national stage of the PCT/JP00/03982 application. Claims 1-13 are pending and are the subject of this Office Action.

DETAILED ACTION

1. Priority

Acknowledgment is made of applicants' claim for priority based on an application filed in Japan on 06/17/99.

2. Objections

2.1. Specification

A substitute specification in proper idiomatic English and in compliance with 37 CFR 1.52(a) and (b) is required. The substitute specification filed must be accompanied by a statement that it contains no new matter.

2.2. Claims

Claims 4 and 11 are objected to for the following reasons. Claim 4 recites the phrase "concentration of zinc ion contained in the culture medium is controlled to 0.1 to 10 mM." The proper phrase is "concentration of zinc ions in the culture medium is 0.1 to 10 mM."

Claim 11 should read: "The process for producing D-aminoacylase according to claim 3, wherein the culture medium contains a tac promoter-inducing substance."

2.3. Drawings

This application has been filed with informal drawings which are acceptable for examination purposes only. Formal drawings will be required when the application is allowed.

3. Rejections

3.1. 35 USC, section 112, second paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claim 1-13 are is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 recites the following phrases:

1) high-expression ability to produce D-aminoacylase,

2) D-aminoacylase producing gene,

3) the expression of the gene product of which is enhanced, and

4) inserting into a host organism.

The phrases are confusing or indefinite rendering the claims indefinite.

Regarding phrase 1), the proper phrase would be "having acquired ability to produce D-aminoacylase". The term "high-expression" is a relative term, which renders the claim indefinite. The term "high-expression" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention.

Regarding phrase 2), a gene does not produce en enzyme. Although we say, "a protein is a product of a gene", a gene encodes the enzyme and the cell containing said gene produces the enzyme.

Phrase 3) it is not grammatical; regarding phrase 4), a microorganism is transformed with a gene. A DNA molecule may be inserted to another DNA molecule.

Dependent claims 2-13 do not correct these errors.

Claim 2 is rejected because the claim recites the term "hybridizing", which renders the claim indefinite. There are many sets of hybridzation conditions in the prior art that are used for DNA molecule selecting by hybridization. The result of the hybridization experiment would vary with the conditions used. Thus, one of skill in the art would not know which conditions to choose. The specification is silent about any hybridization conditions the Applicants intend to use.

Claims 8 and 9 are confusing because of the phrase: "the cell weight of the microorganism either increases or decreases within a range of 10% in a culture medium with 2 mM zinc added thereto on the basis of the cell weight (A660 nm) in a zinc-free culture medium." Measurement of cell culture absorption made for the light with the

wavelength of 660 nm is proportional to the cell density, i.e., to the number of cells per ml, or to the total weight of cells contained in 1 ml of the culture. The correct phrase would be "the density of culture of the host microorganism increases or decreases within a range of 10% in a medium with 2 mM zinc ions per ml, as compared with zinc-free medium." The correct phrase for claim 9 is "the density of culture of the host microorganism increases or decreases within a range of 20% in a medium with 5 mM zinc ions per ml, as compared with zinc-free medium."

2.2. 35 USC, section 112, first paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2.1.1. Lack of written description

Claims 1-13 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are directed to a zinc-tolerant microorganism transformed with a gene encoding D-aminoacylase, wherein the expression of said gene is enhanced in the presence of zinc ions. The claims are directed to a large genus of microorganisms that are zinc-tolerant and contain a gene the expression of which is increased by the presence of zinc ions in the medium. Applicants, however, failed to describe any representative of such a genus, because applicants did not disclose a zinc resistant microorganism containing a D-aminoacylase gene whose expression is increased by the presence of zinc ions. Applicants disclosed a zinc resistant transformant *E. coli* that overexpresses D-aminoacylase of *Alcaligenes xylosoxydans.*

On page 14 Applicants write, "the enzyme activity in the 0.2 mM zinc-added culture medium was 58.86 U/mL (broth –out pH of 5.3) and the enzyme activity in the 2.0 mM zinc–added culture medium was 109.79 U/mL (broth-out pH of 5.11), compared with the enzyme activity of 21.78 U/mL in the zinc-free culture medium (broth-out pH of 5.05). Thus, it has been confirmed that the addition of zinc ion, at least within a predetermined concentration range, greatly improves the D-aminoacylase producing potency".

Under identical conditions of cultivation and measuerments of D-amionacylase activity produced by *Alcaligenes xylosoxydans* the values were 0.12 u/mL, 0.29 U/mL and 0. 29 U/mL, respectively (page 15, line 1). It is important that measurements of the enzyme activity were performed in the full culture medium, i.e. in the medium without zinc or supplemented with zinc, because the enzyme D-aminoacylase is a zinc dependent metalloprotease whose activity is increased in the medium supplemented

with zinc ions (see, for example, the paper by Wakayama et al. Role of conserved histidine residues in D-aminoacylase from *Alcaligenes xylosoxydans* subsp. xylosoxydans A-6", Bioscience, Biotechnology and Biochemistry, 2000, vol. 64, 1-8, on which the Applicants are co-authors; the article is included in the IDS). In case of the medium from *Alcaligenes xylosoxydans* culture, the activity of the enzyme in the presence of 2 mM zinc was the same as in zinc free medium although the growth of the culture was inhibited to 64.6%. A rough estimation indicates that when the medium contains 2 mM zinc the number of molecules of enzyme/mL also decreased at least to 64.6%. Still, 64.6% molecules of the enzyme/mL exhibited 100% activity, which means that the actual activity was 40% higher in the presence of 2 mM zinc.

Data obtained by the applicants for *E. coli* transformants indicate that the activity of D-aminoacylase produced in the cultures supplemented with zinc is higher than in cultures without zinc. These data, however do not prove that the observed increase in the D-aminoacylase activity is a result of the increase in the enzyme expression caused by zinc. The reason is that the applicants did not provide any measurements of the number of D-aminoacylase molecules, or the D-aminoacylase mRNA content per cell of transformant when it was cultivated in the presence or absence of zinc in the medium.

In conlusion, applicants did not provide a sufficient description of the claimed invention so that one skilled in the art was convinced that at the time the application was filed applicants were in possession of the claimed invention.

2.2.2. Scope of enablement

Claims 1-13 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the transformant of zinc resistant E. coli overexpressing D-aminoacylase from *Alcaligenes xylosoxidans* does not reasonably provide enablement for any zinc resistant microorganism transformed with any D-aminoacylase, wherein the expression of the enzyme is increased by the presence of zinc in the medium.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Otherwise, undue experimentation is necessary to make the claimed invention.

Factors to be considered in determining whether undue experimentation is required are summarized *In re* Wands [858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)]. The Wands factors are: (a) the quantity of experimentation necessary, (b) the amount of direction or guidance presented, (c) the presence or absence of working example, (d) the nature of the invention, (e) the state of the prior art, (f) the relative skill of those in the art, (g) the predictability or unpredictability of the art, and (h) the breadth of the claim.

The nature and breadth of the claimed invention encompasses any microorganism resistant to zinc and expressing a D-aminoacylase from any natural or

man-made sources, wherein the expression of said enzyme is increased by the presence of zinc ions in the medium.

Although the art of cloning, and engineering of genes is well developed, and skills of those in the art high, the predictability of the results in selecting a microorganism resistant to zinc, and further transforming it with any D-aminoacylase gene, from any natural or man-made source, so that the expression of said gene was increased in the presence of zinc ions in the culture medium is low. To make and use the claimed invention one skilled in the art is forced to do research outside the realm of routine experimentation. The specification does not disclose any expressing control element whose activity *in vitro* is increased by zinc, nor the specification teaches any expression vector containing a gene encoding D-aminoacylase, wherein said vector after being transected to any zinc resistant microorganism exhibits higher expression when said transformant is cultivated with zinc than without it.

Thus, Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. Without further guidance on the part of Applicants how to make or select an expression controlling element whose activity is increased in the presence of zinc, the experimentation left to those skilled in the art is unnecessary, improperly extensive and undue.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka, Ph.D., whose telephone number

is (703) 305-7270. The examiner can normally be reached Monday-Friday from 10:00 a.m. to 4:30 p.m.

If attempts to reach examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, Ph.D. can be reached on (703) 308-3804. The fax number for this Group is (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionists whose telephone number is (703) 308-0196.

Malgorzata A. Walicka, Ph.D.
Art Unit 1652
Patent examiner

PONNATHAPU ACHUTAMURTHY
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600